Characterization of microparticles derived from cultured macrophages and cerebrospinal fluid of patients with schizophrenic and affective disorders

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Inflammation in the brain can be detected in peripheral blood

Leboyer et al. 2012

- *„Bipolar disorder can be effectively conceptualized as a multi-systemic inflammatory disease“*

Dantzer et al. 2008

- *„When activation of the peripheral immune system continues unabated (...) the ensuing immune signalling to the brain can lead to (...) development of symptoms of depression in vulnerable individuals“*

Hope, et al. 2012

- *„Immune profile of bipolar disorder and schizophrenia suggests inflammatory disturbances related to neuroplasticity, endothelial function and calcium regulation“*

Scientists have known for a while now that inflammation contributes to long-term neurodegenerative conditions such as AD and PD. But lately they have been turning up evidence that *inflammation* can affect the brain more directly and acutely, and might underlie a wider range of problems, from impaired cognition during infections to *depression* and even *schizophrenia*. - See more at: http://www.dana.org/BrainWork/2014/The_Brain_Inflamed/#sthash.QFa2Orpw.dpuf

Patients

Case	Age[ ], Male/female	Disease	Autoimmune characteristics	Medication A, B, C, D, F
SZ1	[25] male	F20.0	no	B, B, F
BD1	[47] female	F32.2	yes	D, F
BD2	[51] female	F32.2	yes	C, F, B
SZ2	[33] female	F20.0	yes	none
BD3	[40] male	F42.2 F60.7	no	none
BD4	[28] male	F33.2 F60.3	yes	B, Bupropione 300 mg
BD5	[51] male	F.45.0	no	Bupropione 200 mg + Amitriptyline 25 mg
BD6	[31] male	F42.2	yes	B, Antibiotics
BD7	[41] male	F06	no data	no data
BD8	[28] female	F43.0	no	no data
SZ3	[59] female	F20.0	no	B, B

Aims

To characterize inflammatory pathways in enriched antigen presenting cells

To clarify the involvement of
damage vs. pathogen related inflammation

Aims

Dangers

Strangers

Methods

Whole blood→Ficoll separation→culture of plastic adherent cell fraction [<28 days]
↓
Flow cytometry: Cells and microparticles
↓
Enrichment of microparticles
↓
Electron microscopy
↓
miRNA quantification

Reverse phase HPLC
↓
Kynurenin/tryptophan ratio

Transelectron Microscopy Metabolism

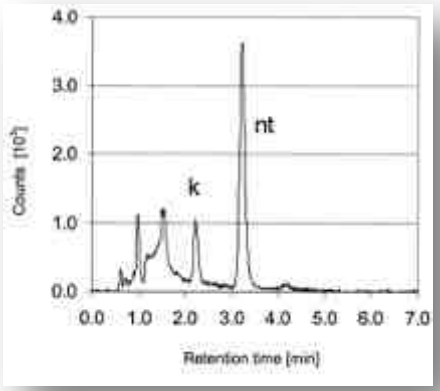
Cell enrichment→chemical fixation
↓
microscopy

High Pressure Liquid Chromatography (HPLC)
↓
indoleamine 2,3-dioxygenase (IDO), tryptophan, nitric oxide

Autophagy
Apoptosis
Necrosis
Pyroptosis
Necrosis

Microparticles
Infectious agents

Kynurenine per tryptophan ratio /nitic oxide
↓
Activation of IFN-γ→TH1, NK cell activation

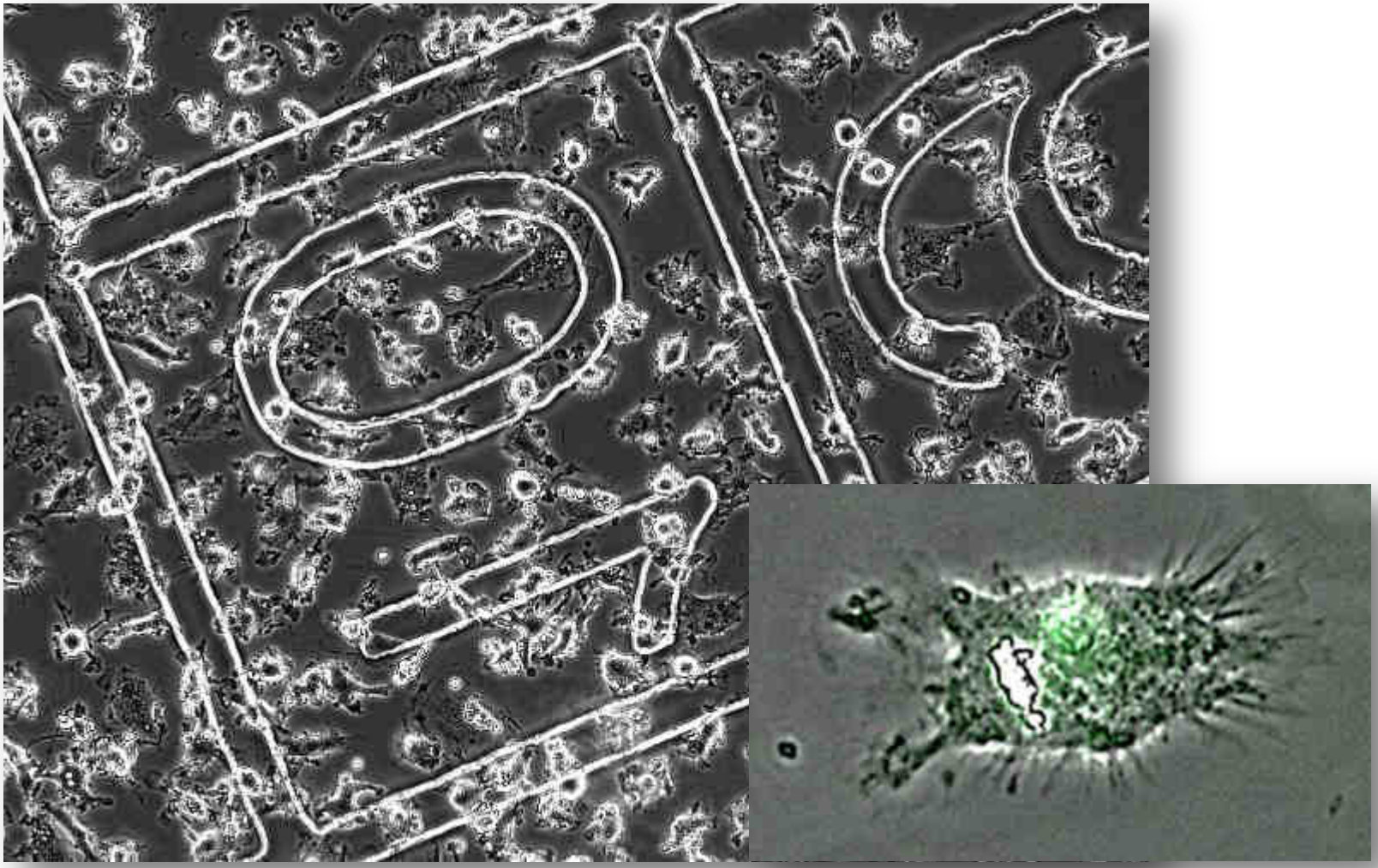


ELISA for biomarkers

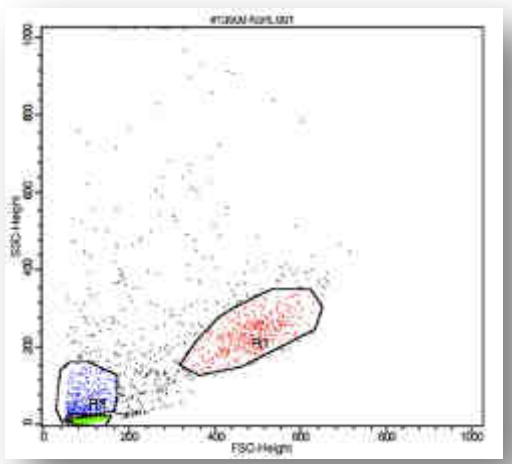
D. Fuchs, *Clin Chem 2002 vol. 48 (3) p.3 579-581*

Cell Culture

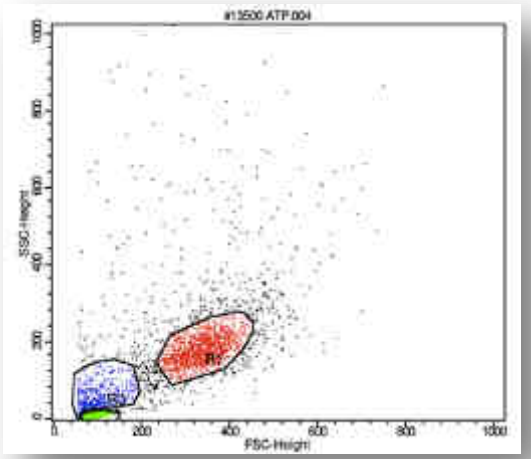
plastic adherent cell fraction [<28 days]

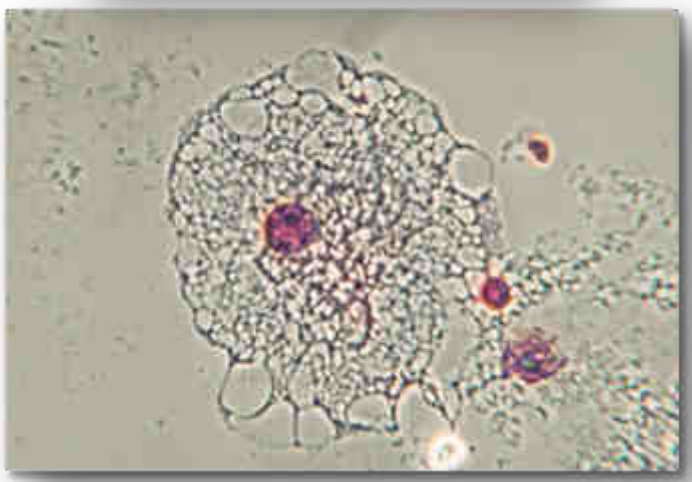


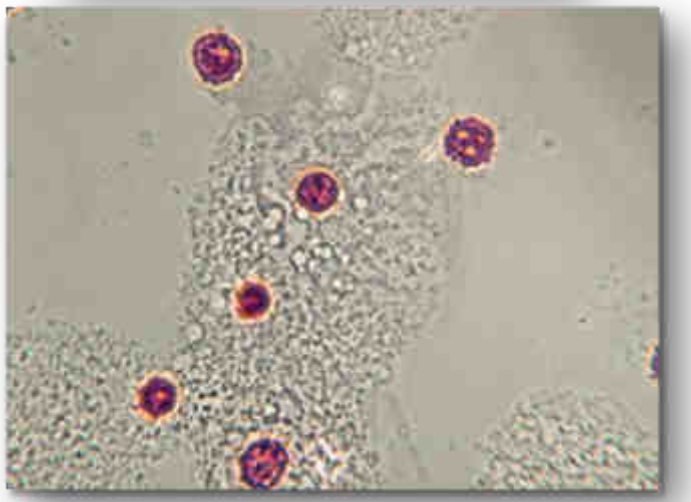
ATP stimulation induces microparticle release



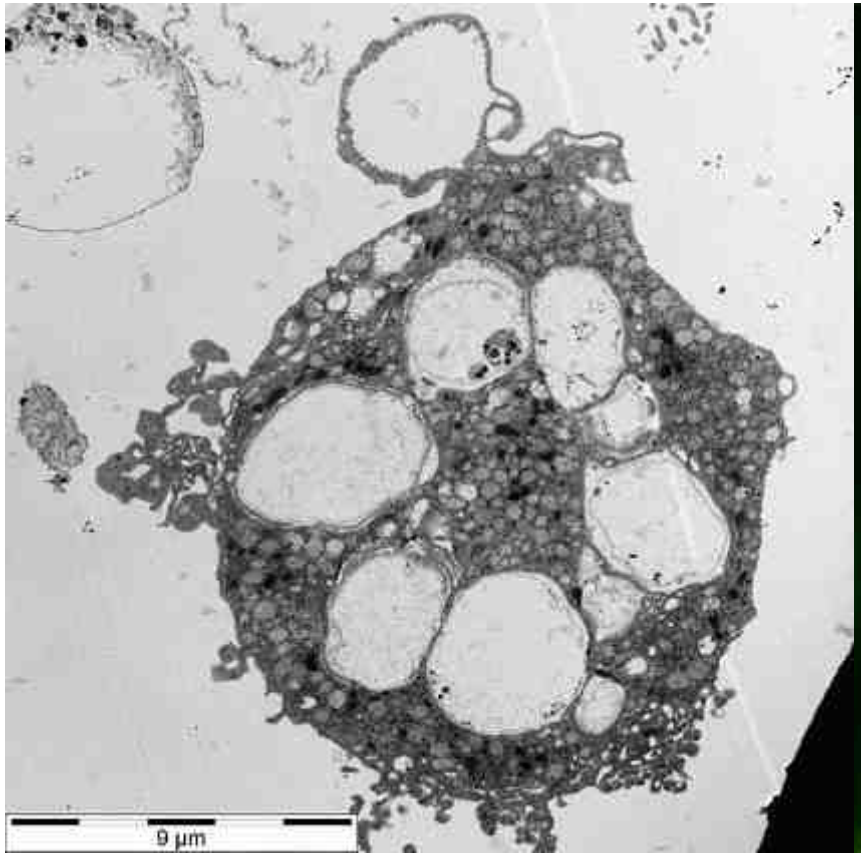
1 mM ATP

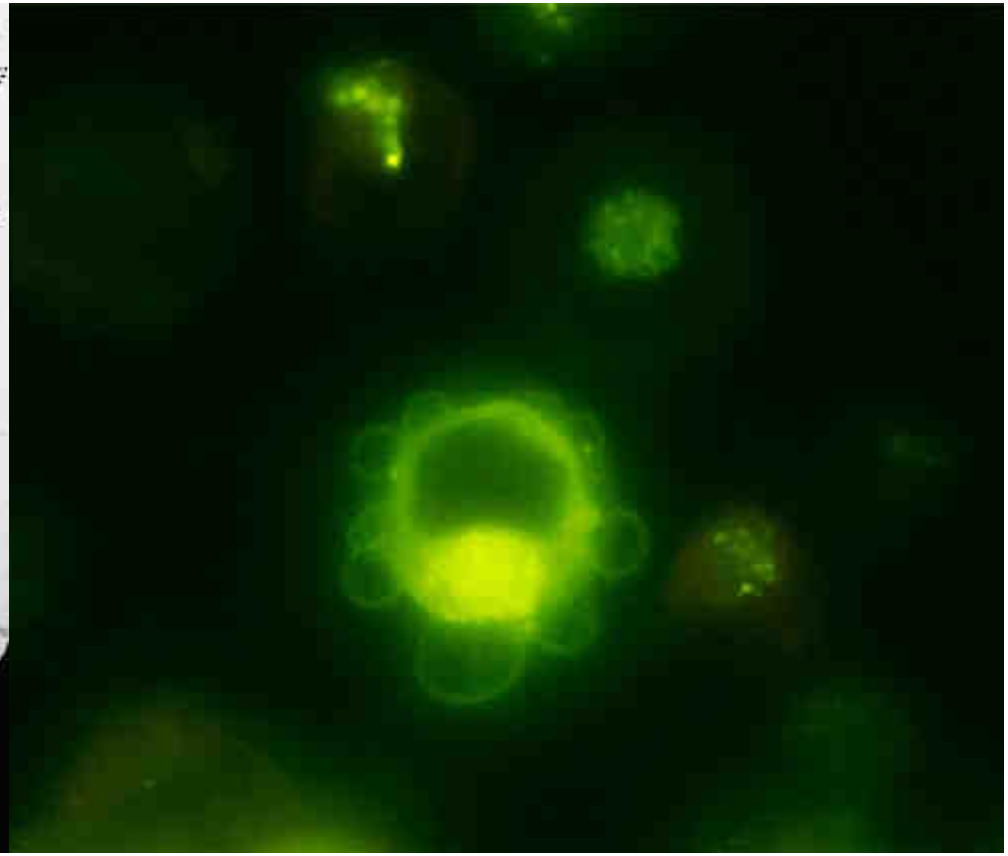




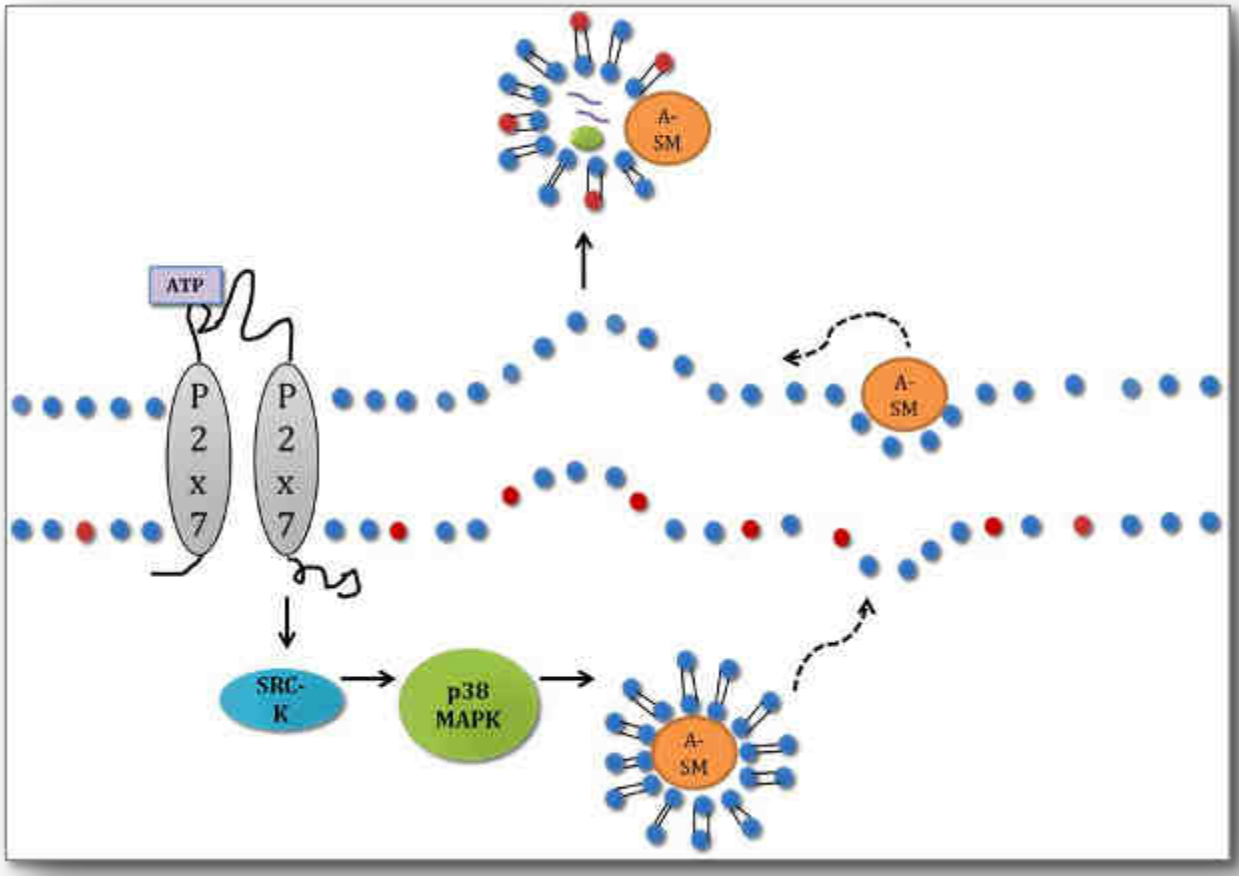


Microparticles are derived from the plasma membrane



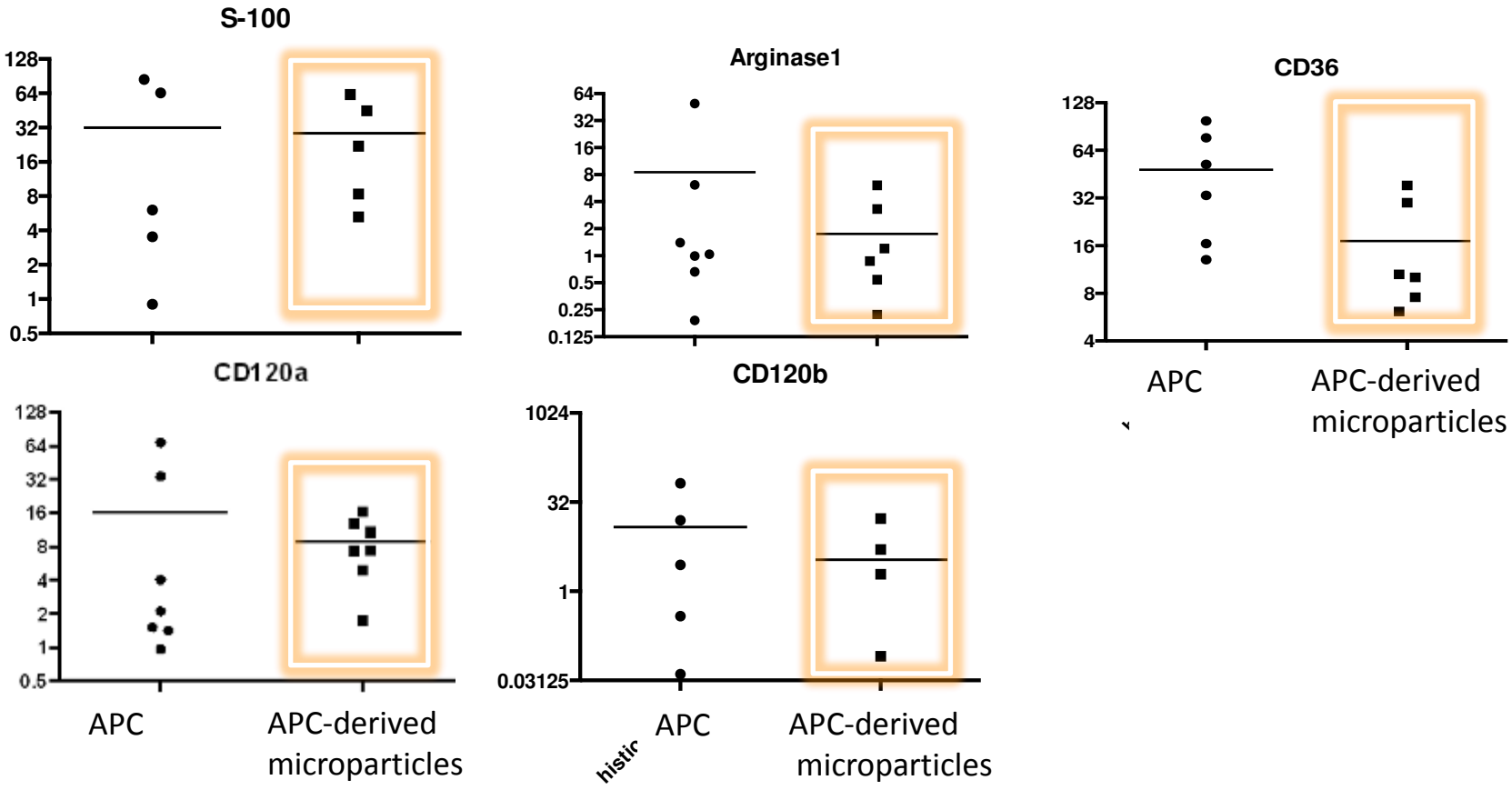


Mechanism of ATP induced microparticle release

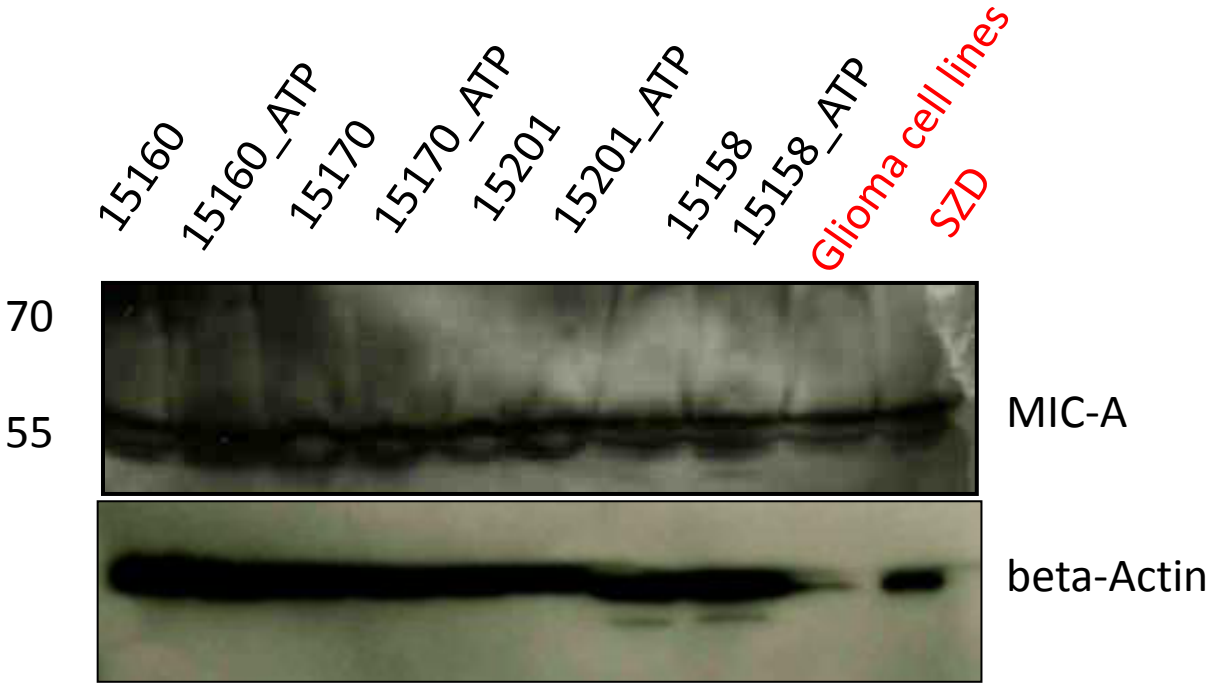


Bianco, F. et al. EMBO J 2009; 28, 1043-1054

Microparticles are derived from the plasma membrane

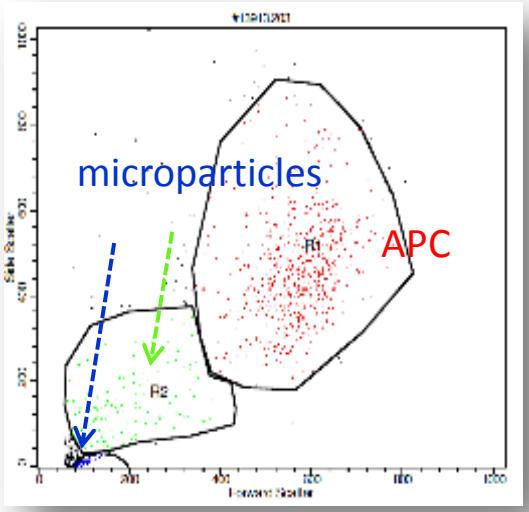


Western Blot of MPs prepared from cultured antigen-presenting cells (APC) following ATP stimulation

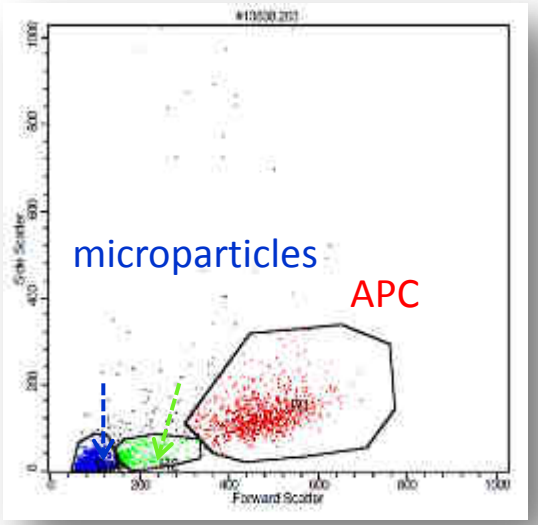


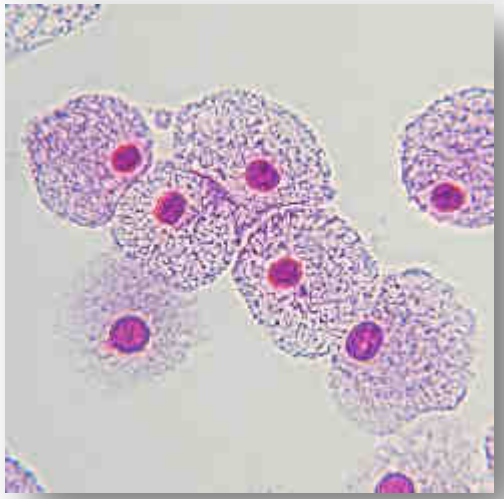
Phenotype analysis

iDC



MΦ

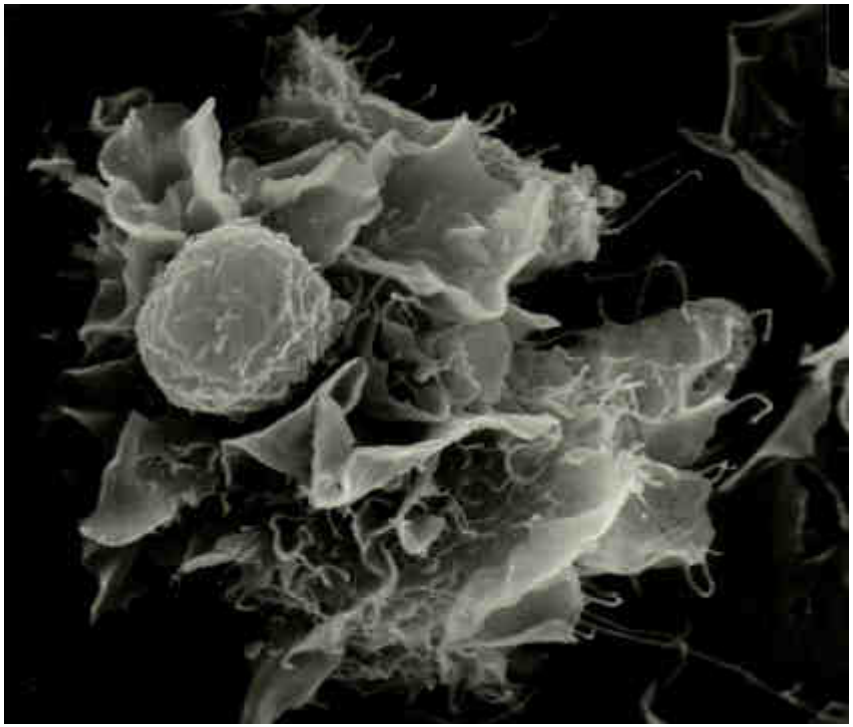




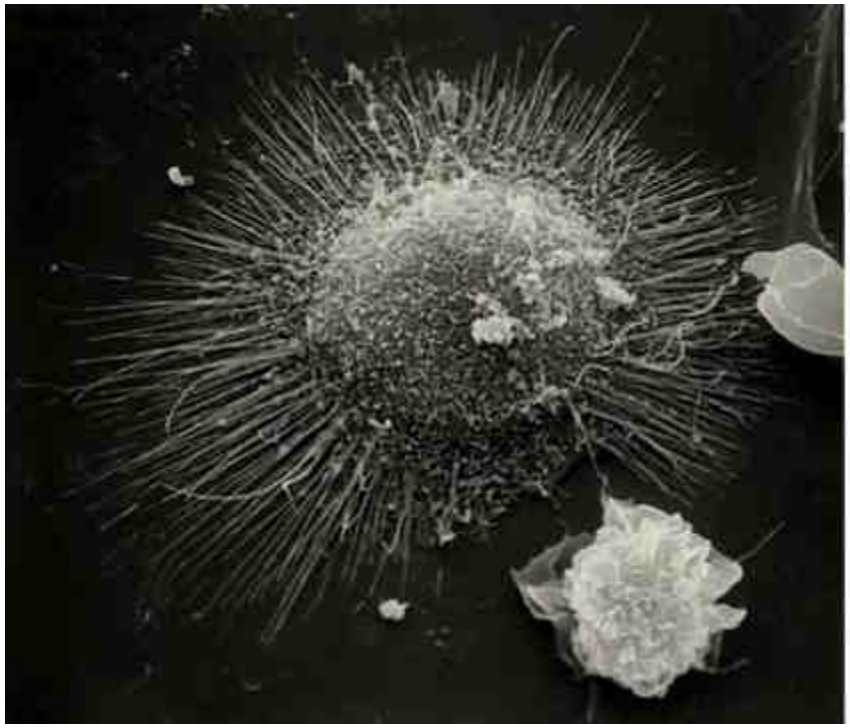
15794

Morphology

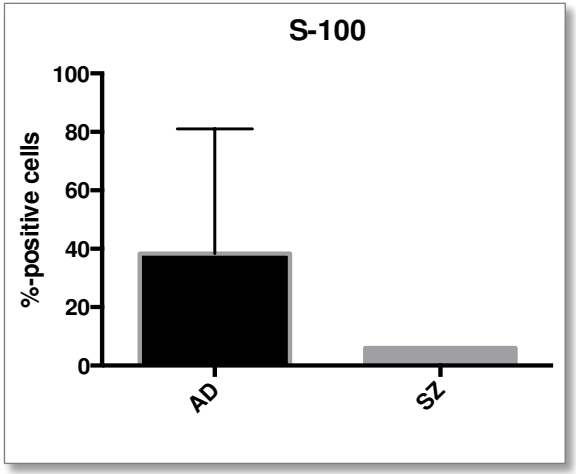
M1

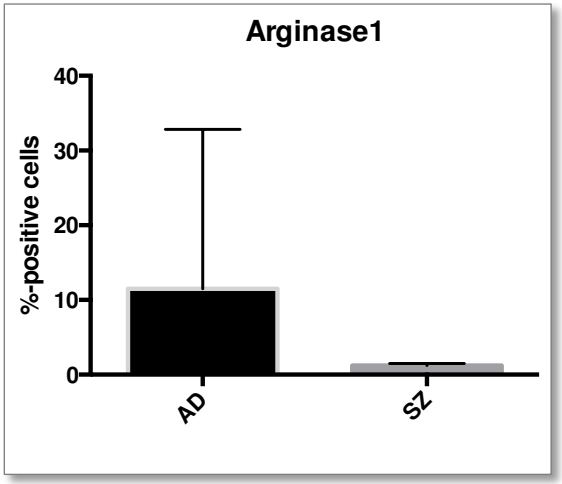


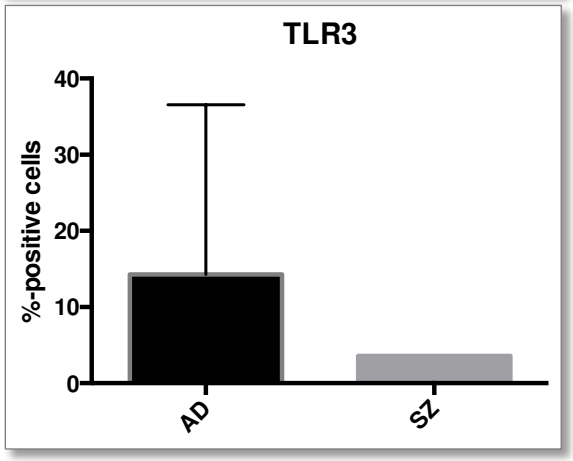
M2

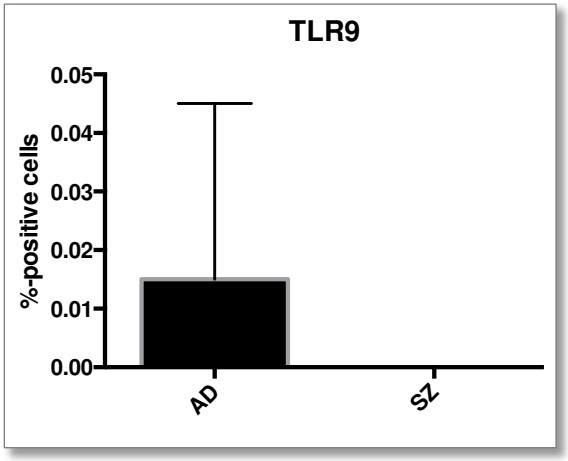


M1/M2 phenotype in AD (affective disorder) and SZ (schizophrenia)

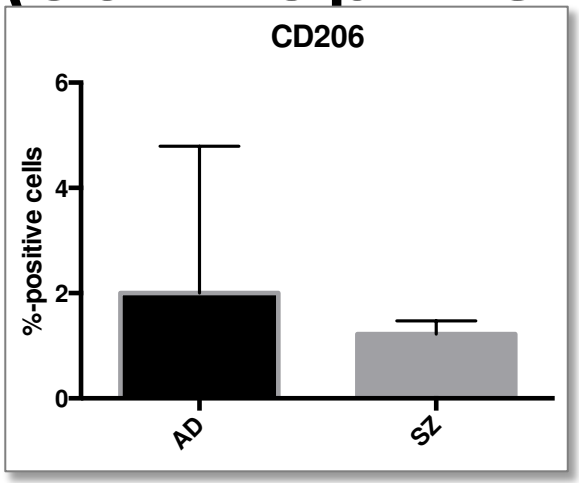


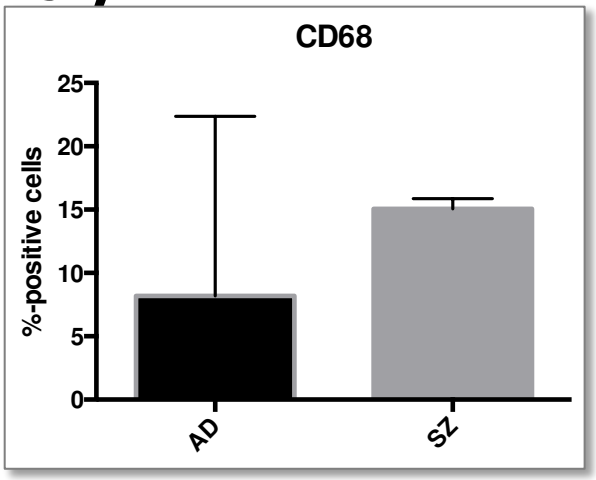


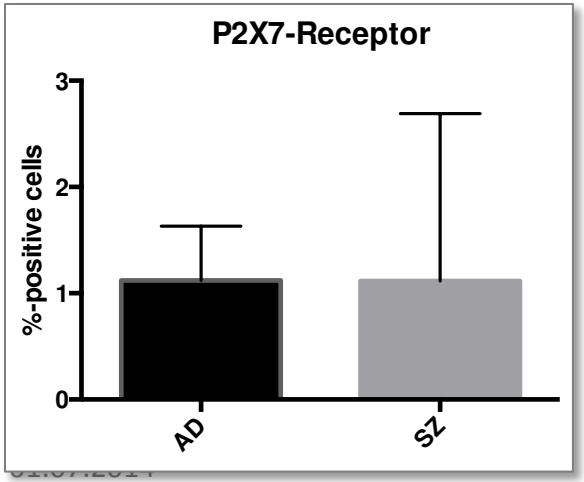


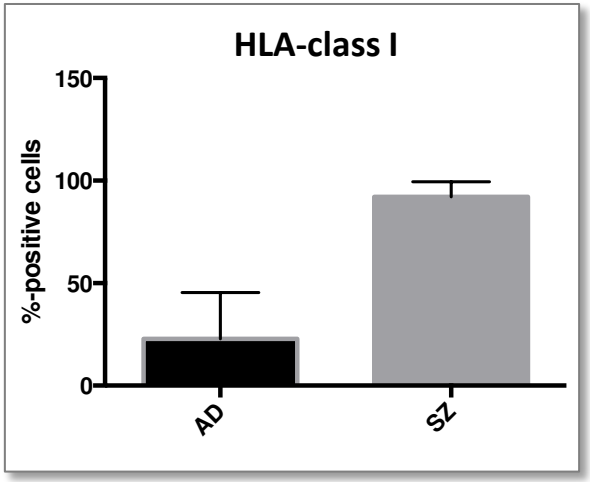


M1/M2 characteristics in AD (affective disorders and SZ (schizophrenia)

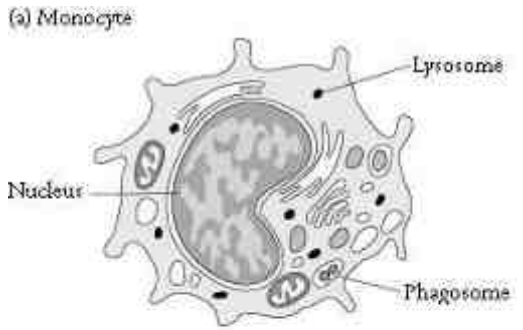


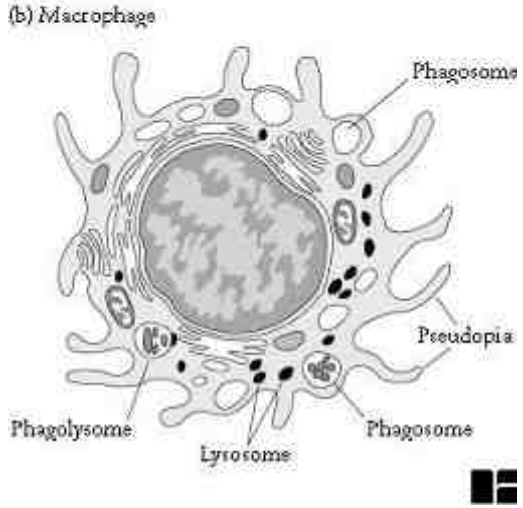


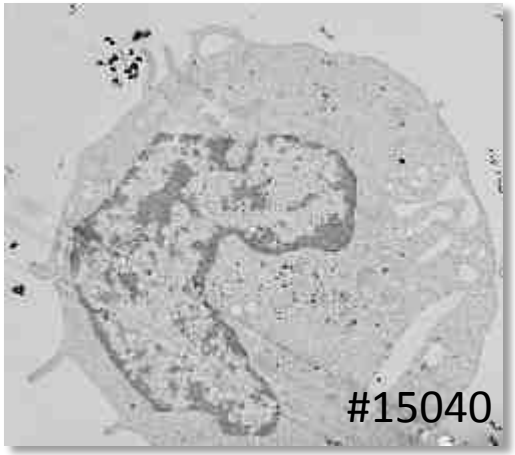


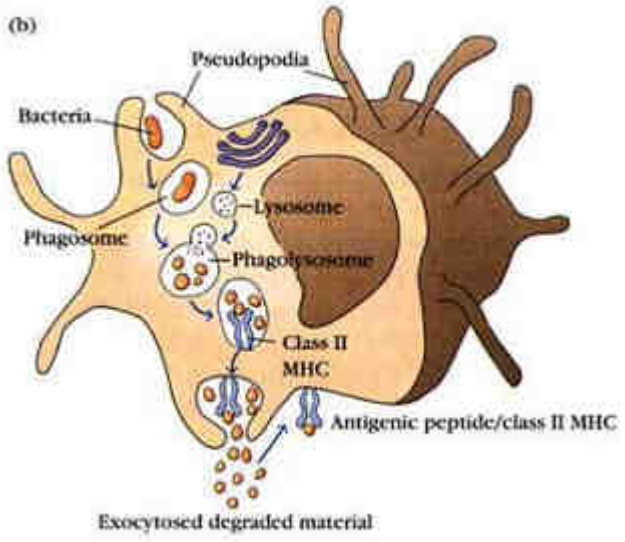


Cell types to be detected in CSF



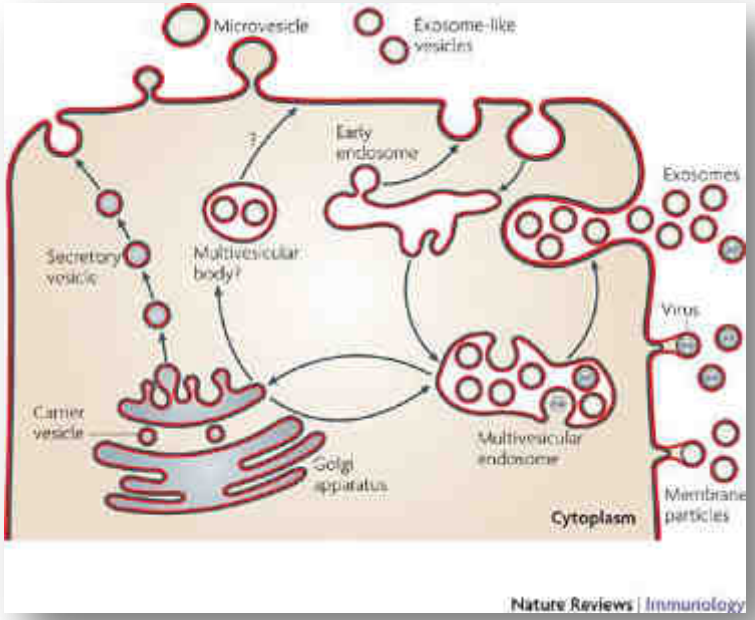




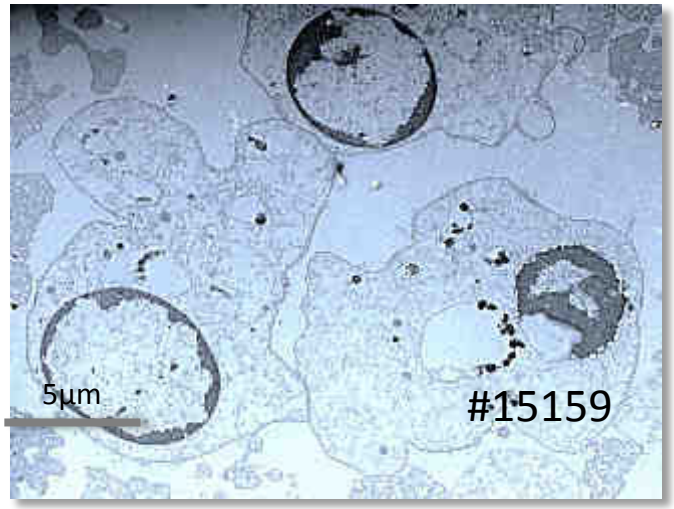


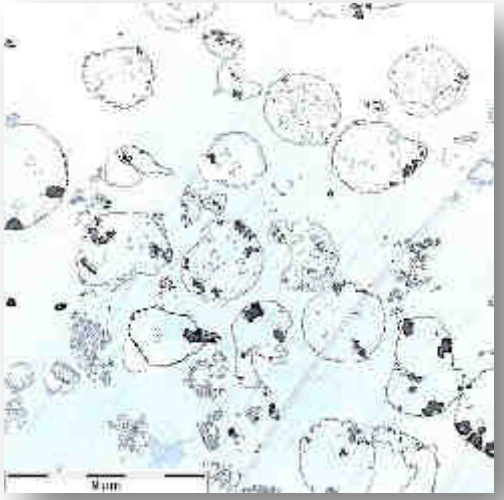
http://www2.hawaii.edu/~johnb/micro/medmicro/medmicro.7.html

Cell derivatives to be detected in CSF

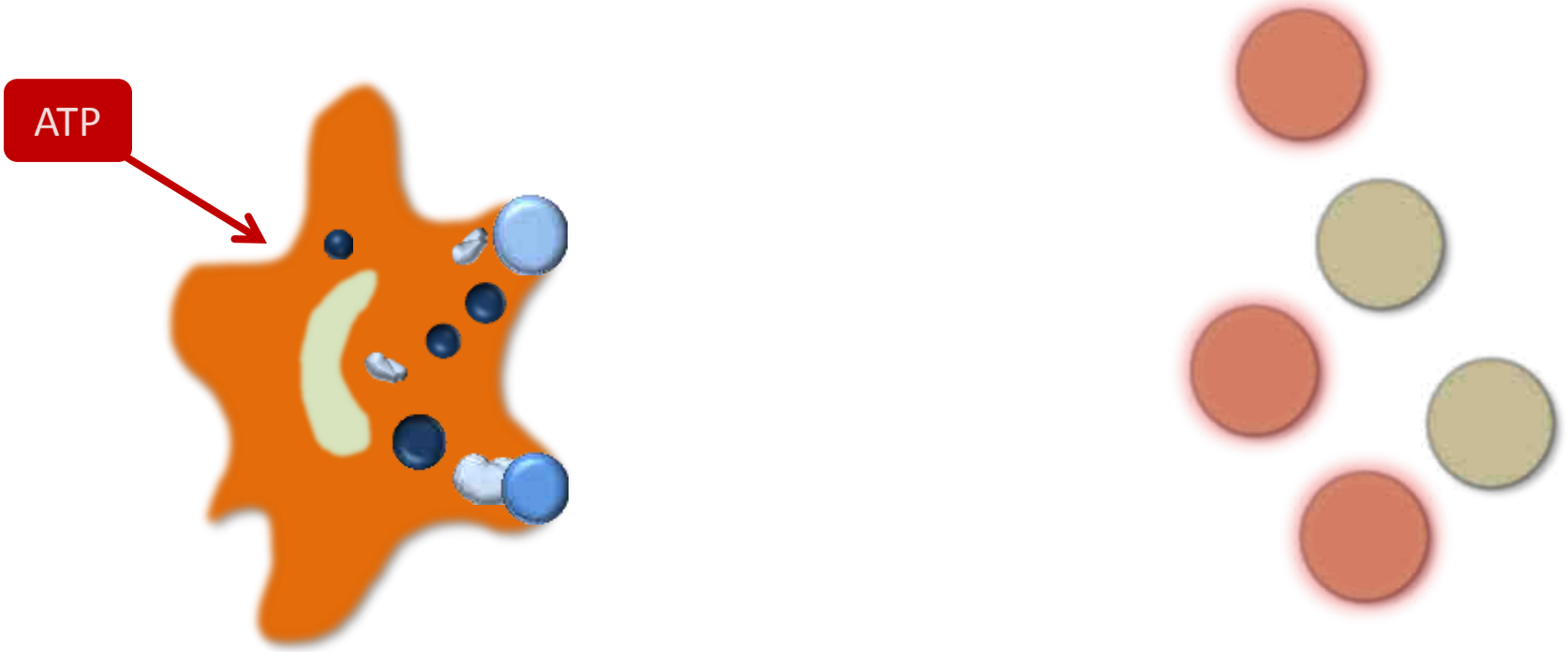


Clotilde Théry et al Nature Rev Immunol. 2009; (9) 581-593

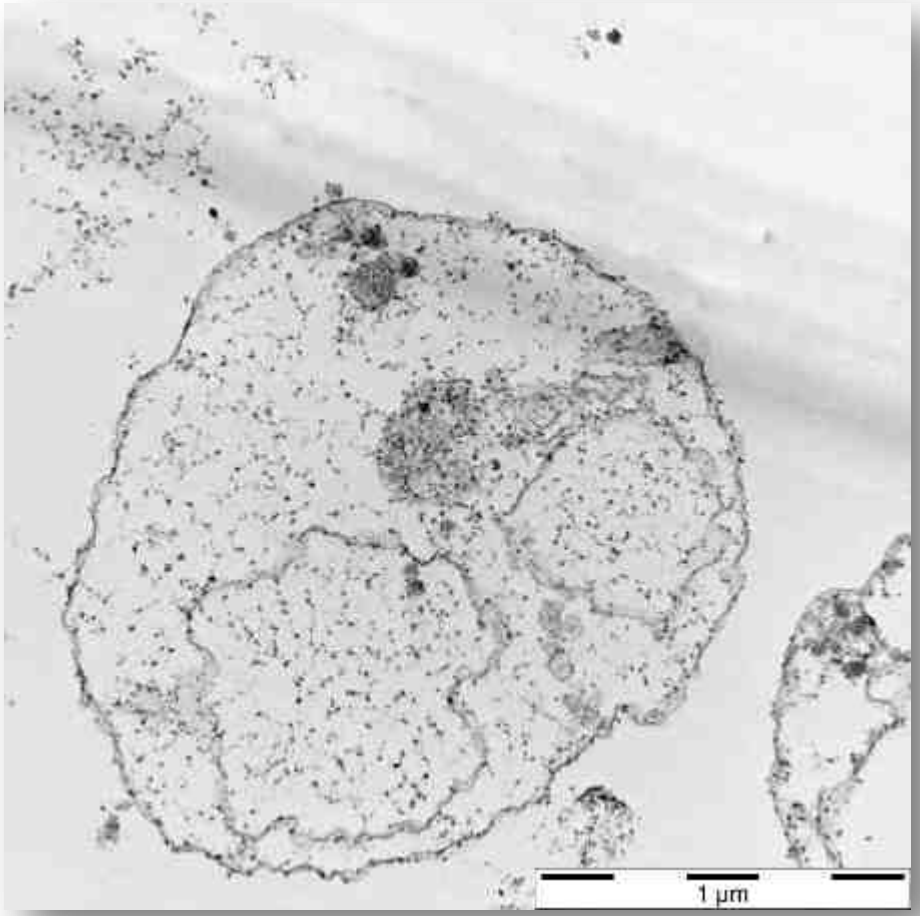




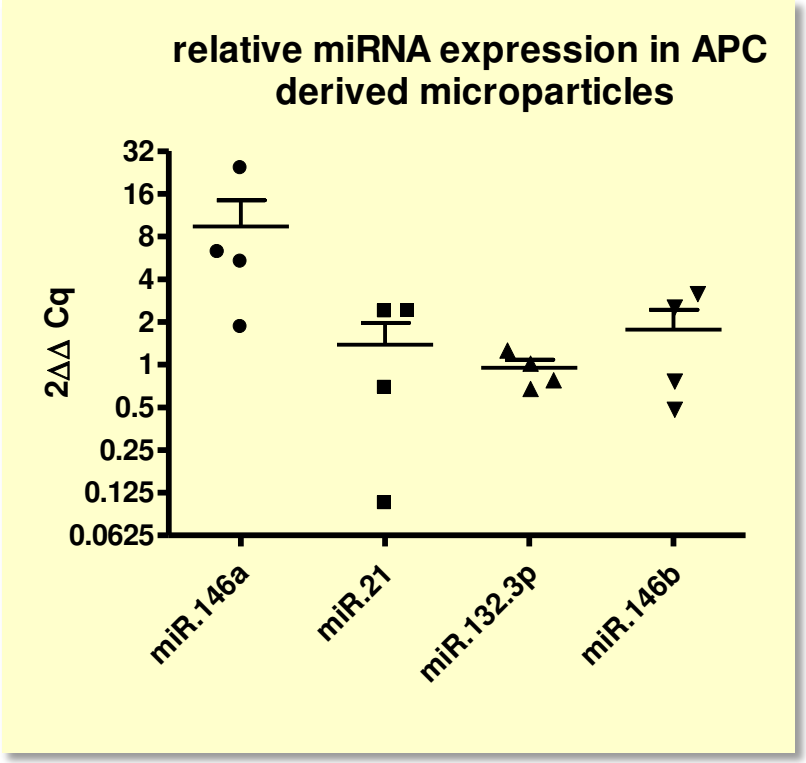
ATP induced release of microparticles and target cell fusion



Typical microparticle derived from cultured antigen presenting cells



miRNA expression in ATP-induced MP



Four patients‘ cultured antigen presenting cells were stimulated with ATP and qPCR was performed for the expression of miRNA species. All patients suffered from AD or SZ.

Summary

An inflammatory phenotype of in vivo activated APC in AD and SZ has been shown.
Prominent release of MPs from cultured APC occurs upon ATP-induced P2X7 ion channel activation

MPs are derived from the plasma membrane
MPs transport miRNA species related to inflammation and stress or infection induced cognitive impairments